PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 257/02, 401/06, 207/26, C07C 235/06, 235/10, A61K 51/04

A1

(11) International Publication Number: WO 95/01346

(43) International Publication Date: 12 January 1995 (12.01.95)

(21) International Application Number: PCT/EP94/02126

(22) International Filing Date: 28 June 1994 (28.06.94)

(30) Priority Data:
93201902.9 30 June 1993 (30.06.93) EP
(34) Countries for which the regional or international application was filed: NL et al.

(71) Applicant *(for all designated States except US)*: AKZO NOBEL N.V. [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KASPERSEN, Franciscus, Michael [NL/NL]; Acacialaan 13, NL-5384 BB Heesch (NL). REINHOUDT, David, N. [NL/NL]; De Voskamp 7, NL-7552 GD Hengelo (NL). VERBOOM, Willem [NL/NL]; De Nachtegaal 6, NL-7671 WB Vriezenveen (NL). VAN STAVEREN, Catherina, Joanna [NL/NL]; Zevenblad 17, NL-5345 KX Oss (NL).

(74) Agent: BEETZ, T.; Postbus 20, NL-5340 BH Oss (NL).

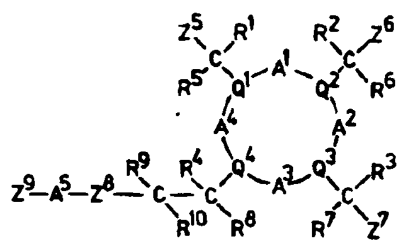
(81) Designated States: AU, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CHELATING COMPOUNDS

(57) Abstract

1.



(1)

The invention provides chelating comounds having nine ligand sites capable of simultaneously chelating a single metal cation as well as their metal chelates, the compound having formula (1), wherein A^1, A^2, A^3, A^4 and A^5 represent optionally substituted organic chains; Q^1, Q^2, Q^3 and Q^4 each represent a nitrogen atom, or Q^1, Q^2, Q^3 and Q^4 represent C-Z^1, C-Z^2, C-Z^3 and C-Z^4 respectively, in which Z^1, Z^2, Z^3 and Z^4 are exocyclic and represent O, S or NR^{11}; Z^5, Z^6 and Z^7 preferably represent carboxyl; Z^8 represents O, S or NR^{12}; Z^9 represents O, S or NR^{13}, to which a hydrogen atom or an organic substituent may be attached; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} independently represent hydrogen, C_1-C_6 alkyl or C_2-C_6 alk(adi)enyl, or any pair of R^1 and R^5, R^2 and R^6, R^3 and R^7, R^4 and R^8, and R^9 and R^{10} may also represent oxo, or R^8 and R^9 or R^9 and a substituent at A^5 may be connected to form a ring; R^{11} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl or C_1-C_6 hydroxyalkyl; R^{12} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 hydroxyalkyl or a second bond together with one of the neighbouring chain atoms of Z^8; R^{13} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 hydroxyalkyl, ar(alk)yl or a second bond with the neighbouring chain atom of Z^9; wherein one of R^1, R^2, R^3, R^4 and R^9 or one of the susbtituents on A^1, A^2, A^3, A^4, A^5 or Z^9 may represent A^6-X, wherein A^6 represents a direct bond or an organic linking group, and X represents a functional group capable of binding to a targeting molecule; or a mono- or polyanion thereof derived by abstraction of one or more acidic hydrogens.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

Chelating compounds

The present invention relates to chelating compounds, metal chelates and conjugates of these chelates with targeting biomolecules for use as targeted (radio)diagnostic and (radio)therapeutic agents.

Chelating compounds are useful in medicine as diagnostic agents and therapeutic agents for the controlled supply of metals, e.g. in nuclear magnetic imaging, radioimmunoscintigraphy and radioimmunotherapy. Metals to be used can be stable or radioactive, depending on the type of usage. Chelating compounds that are covalently bound to targeting molecules, such as antibodies, can be regarded as bifunctional chelators capable of conveying a metal to a specific site in the body, for therapeutic or diagnostic purposes, e.g. to bring a radionuclide to a tumour cell for locating or treating the tumour.

US 5,053,503 discloses bifunctional chelating agents which are or 1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetraacetic acid (DOTA) or 1,4,7,10-tetraaza-cyclododecane-1,4,7-triacetic acid (DO3A), containing a function capable of reacting with a protein site, as well as radiodiagnostic and radiotherapeutic agents containing such a chelating agent, a metallic radionuclide and an antibody. Similarly, WO 89/01476 describes DOTA having a functional group, in particular a 6-vinyl-2-pyridylmethoxy-acetamido-butyl group, linked at the 2-position of DOTA. EP-A-292,689 discloses DO3A derivatives having a substituent with a terminal functional group at N-10; as an example 10-(N-hydroxyethylcarbamoyl-methyl)-DO3A is described. EP-A-374,947 describes DOTA derivatives having a aminophenyl or isothiocyanatophenyl groups as coupling functions attached at one of the cyclododecane carbon atoms or at the α-position of one of the acetic acid residues.

EP-A-434,346 discloses 10-(3-polyethyleneoxy-2-hydroxypropyl)-DO3A and its metal complexes as diagnostic contrast agents. Similarly, 10-poly(oxyethylene)-DO3A derivatives are disclosed in EP-A-466,200 and 10-trihydroxybutyl-DO3A derivatives are described in EP-A-448,191. EP-A-287,465 discloses, inter alia, 2-hydroxyethyl-DOTA and 1,4,7,10,13-penta-aza-cyclopentadecane-1,4,7,10,13-pentaacetic acid as well as their metal complexes for use in radiodiagnostics. EP-A-325,762 discloses, inter alia, α-hydroxymethyl-1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetra-acetic acid, its mono(hydroxyalkyl)amides and their metal chelates as radiodiagnostic agents. Chelating compounds wherein one or two 1,4,7,10-

tetraaza-cyclododecane-1,4,7-triacetic acid groups are bound at their 10-position to a heterocycle are described in WO92/12978.

Calix[4]arenes, which are phenol-formaldehyde cyclic tetramers, having four carbamoylmethoxy substituents at the 2-positions of the phenyl rings, and their complexes with lanthanide ions have been described by Sabbatini et al., J. Chem. Soc. Chem. Comm. 1990, 878-9.

There is a need for improved (radio)diagnostic and radio-therapeutic agents comprising a (radioactive) metal and a targeting agent (e.g. an antibody), wherein the metal is tightly bound with the targeting agent. This requires a chelating compound which complexes the metal ions very rapidly under mild, ambient conditions (such as temperatures from 20 to 40°C and a pH between 4 and 8), even at very low concentrations of the metal ions, in order to allow an effective and economical use of the reagents. Also, decomplexation must be very slow so as to reduce the release of metal ions, e.g. in the human body, at the acceptable minimum, preferably well below 1% per day. The chelating agents known e.g. from the references cited above, although satisfactory in some applications, do not meet these high standards.

The object of the present invention is thus to provide chelating (complexing) compounds that can rapidly form highly stable complexes with metal ions, in particular trivalent radioactive metal ions. A special object of the invention is to provide such chelating compounds which also have a function capable of binding to a targeting agent.

The object is achieved according to the invention by means of chelating compounds which in one and the same molecule have nine ligand sites preorganised in the molecule in such a way that they are capable of simultaneously chelating a single metal cation. The nine ligand sites of these chelating compounds are selected from nitrogen, oxygen and sulphur atoms having a free pair of electrons. The compounds preferably contain a functional group capable of binding to a targeting agent.

In particular the chelating compounds according to the invention are represented by formula 1, wherein:

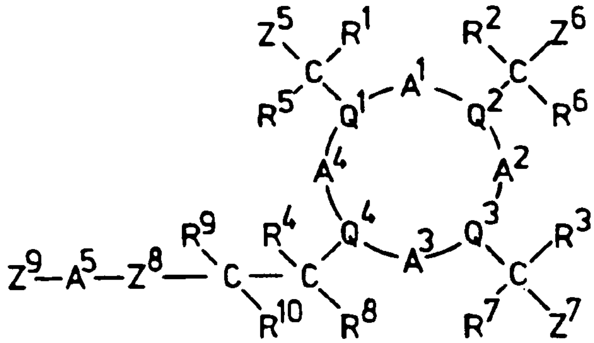
A^1, A^2, A^3 and A^4 represent optionally substituted organic chains;

A^5 represents an optionally substituted organic chain;

Q^1, Q^2, Q^3 and Q^4 represent Z^1, Z^2, Z^3 and Z^4 respectively, in which case each of these is a nitrogen atom, or Q^1, Q^2, Q^3 and Q^4 represent $C\text{-}Z^1$, $C\text{-}Z^2$, $C\text{-}Z^3$ and $C\text{-}Z^4$ respectively, in which case Z^1, Z^2, Z^3 and Z^4 are exocyclic and independently represent O, S or NR^{11}; wherein the spatial

distances between Z^1 and Z^2, Z^2 and Z^3, Z^3 and Z^4, and Z^4 and Z^1 each can be 3.15 ± 0.25 Å, and the spatial distances between Z^1 and Z^3, Z^2 and Z^4 each can be 4.45 ± 0.35 Å;

1.



Z^5, Z^6 and Z^7 independently represent carboxyl, thiocarboxyl, carboximidoyl, phosphono, thiophosphono, phosphorimidoyl, sulpho, thiosulpho, sulfamoyl, preferably carboxyl or thiocarboxyl;

Z^8 represents O, S or NR^{12};

Z^9 represents O, S or NR^{13}, to which a hydrogen atom or an organic substituent may be attached;

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} independently represent hydrogen or C_1-C_6 alkyl or alk(adi)enyl, or any pair of R^1 and R^5, R^2 and R^6, R^3 and R^7, R^4 and R^8, and R^9 and R^{10} may also represent oxo, or R^8 and R^9 or R^9 and a substituent at A^5 may be connected to form a ring together with the carbon atoms to which they are bound;

R^{11} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl or C_1-C_6 hydroxyalkyl;

R^{12} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 hydroxyalkyl or a second bond together with one of the neighbouring chain atoms of Z^8;

R^{13} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 hydroxyalkyl, optionally substituted C_5-C_{10} aryl or C_6-C_{14} aralkyl, or a second bond together with the neighbouring chain atom of Z^9;

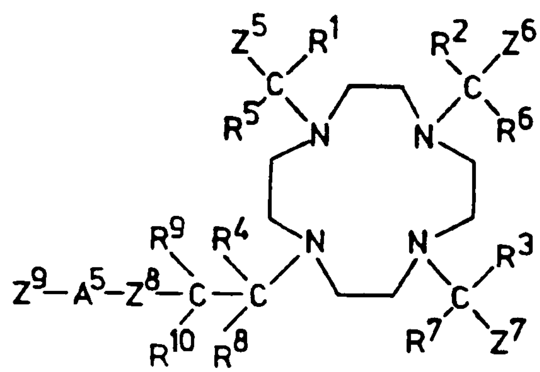
wherein one of R^1, R^2, R^3, R^4 and R^9 or one of the substituents on A^1, A^2, A^3, A^4, A^5 or Z^9 may represent A^6-X, wherein A^6 represents a direct bond or an organic linking group, and X represents a functional group capable of binding to a targetting molecule;

or a mono- or polyanion thereof derived by abstraction of one or more acidic hydrogens;

with the proviso that if Q^1, Q^2, Q^3 and Q^4 are each nitrogen and Z^9 is hydroxy, $C\text{-}R^9R^{10}\text{-}Z^8$ does not represent methyleneoxy or carbonylimino.

In a variant of the compounds according to the invention, the symbols Q^1, Q^2, Q^3 and Q^4 each represent a nitrogen atom as Z^1, Z^2, Z^3 and Z^4, and together with the organic chains A^1, A^2, A^3 and A^4 they constitute a tetraaza-cycloalkane system, as shown in formula 3.

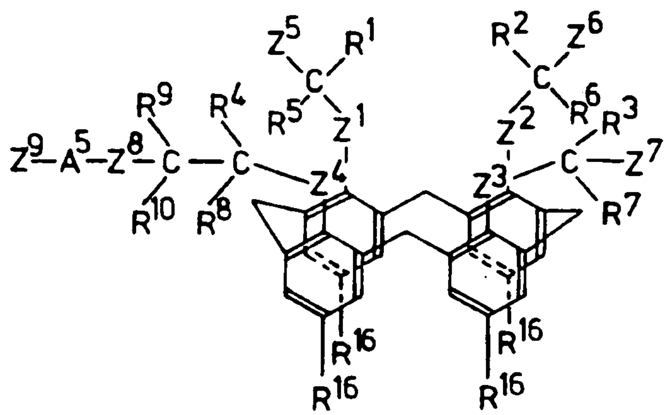
3.



In the compounds having formula 3, the organic chains A^1, A^2, A^3 and A^4 preferably represent two-carbon or three-carbon chains, most preferably two-carbon chains. The corresponding ring systems include 1,4,7,10-tetraaza-cyclododecane, 1,4,7,10-tetraaza-cyclotridecane and 1,4,8,11-tetraaza-cyclotetradecane. The organic chains may also be unsaturated and/or substituted, e.g. with C_1-C_6 alkyl groups, resulting in e.g. the cyclododecene, cyclotetradecadiene, 2,5,8,11-tetramethyl-cyclododecane, and dibenzo[*b,h*]cyclododecadiene analogues of these tetra-azacycloalkane systems. One of the four organic chains, e.g. A^1 or A^3, may advantageously substituted with A^6-X, i.e. with the group providing a bonding function for a targeting agent. The preferred ring system is 1,4,7,10-tetraaza-cyclododecane.

In another variant of the compounds according to the invention, the symbols Q^1, Q^2, Q^3 and Q^4 represent $C\text{-}Z^1$, $C\text{-}Z^2$, $C\text{-}Z^3$ and $C\text{-}Z^4$ respectively, wherein the C-atoms are inserted between A^1, A^2, A^3 and A^4, and each have a double bond with one of the neighbouring ring atoms. In particular, each C-atom together with both its neighbouring ring atoms forms a benzene ring thus constituting a tetrabenzo[*ab,ef,ij,mn*]cyclo-hexadecatetraene. Z^1, Z^2, Z^3 and Z^4 represent O, S or NR^{11}, preferably O, attached at positions 2, 6, 10 and 14 of the cyclohexadecatetraene as shown in formula 2.

2.



The benzo groups may contain a p-substituent R^{16} such as C_1-C_6 alkyl, in particular tert-butyl, whereas one of the four symbols R^{16} may also represent the bonding group A^6-X as described above.

In the formulae 1, 2 and 3, the R^1, R^2, R^3 and R^4 preferably represent hydrogen or C_1-C_6 alkyl, whereas one of the three may also represent the group A^6-X, and R^5, R^6, R^7 and R^8 preferably represent hydrogen. Most preferably each of R^1, R^2, R^3, R^4, R^5, R^6, R^7 and R^8 represents hydrogen.

R^9 and R^{10} may advantageously together represent an oxo group, especially when Z^8 is a nitrogen group NR^{12}. Also, each one of these symbols R^9 and R^{10} may represent hydrogen or one hydrogen and one C_1-C_6 alkyl or C_2-C_6 alk(adi)enyl, in which cases R^4 and R^8 may be hydrogen or together be oxo. As another useful alternative, if Z^8 is a preferred nitrogen group NR^{12}, R^9 may be connected with R^{12}, or with the first atom of the chain A^5 to form a ring such as pyridine, in which latter case R^{12} represents a double bond with one of the neighbouring carbon atoms.

The group A^5 is preferably a two-carbon or three carbon chain that may be substituted. The substitution may be so as to form a ring. In particular (Z^8)-A^5-(Z^9) may represent a group (Z^8)-$CR^{17}R^{18}$-$(CHR^{21})_n$-$CR^{19}R^{20}$-(Z^9), wherein n equals 0 or 1, R^{17}, R^{18}, R^{19}, R^{20} and R^{21} independently represent hydrogen, C_1-C_6 alkyl or C_2-C_6 alk(adi)enyl, and R^{17} and R^{18}, or R^{19} and R^{20} may also together represent oxo, and wherein R^9 and R^{17}, R^{12} and R^{17}, R^{17} and R^{19}, R^{17} and R^{21}, R^{19} and R^{21}, R^{19} and R^{13}, or R^{19} and the substituent attached to Z^9 may also be connected to form a ring. One of R^{17}, R^{18}, R^{19}, R^{20} and R^{21} may also represent the group A^6-X.

The group Z^9 may e.g. be hydroxy, alkoxy, mercapto, alkylthio, amino, (di)alkylamino, acylamino, or may be a part of a heterocycle which

does or does not include the neighbouring atom of A^5. Z^9 may advantageously carry the group A^6-X. Examples of the combined group A^5-Z^9 are carboxymethyl, 3-(2-oxo-1-pyrrolidinyl)propyl, acetylaminoethyl, 2-pyridylmethyl, N-(2-mercaptoethyl)carbamoylethyl, and 2-pyridylacetyl; examples of the combined groups CR^9R^{10}-Z^8-A^5-Z^9 are 6-hydroxymethyl-2-pyridyl, 6-carboxy-2-pyridyl and 6-(benzylcarbamoyl)-2-pyridyl.

In the group A^6-X, X may e.g. represent isocyanato, isothiocyanato, mercapto, amino, haloacetyl, diazo, succinimido, maleimido, a reactive ester group or an anhydride. A^6 may be any organic chain which may incorporate alkylene, alkenylene, cycloalkylene, arylene, carbonyl groups, heteroatoms; A^6 may for example be or contain ethylene, butylene, methylenecarbonyl, polyoxyethylene, phenylene, phenylenemethylene etc. The combined groups A^6-X may be e.g. be ω-mercaptoalkyl, p-aminophenyl, p-isothiocyanatobenzyl, p-aminophenylalkyl, chloroacetylmethyl, p-diazoniobenzyl, ω-maleimidoalkyl, etc. The linker A^6 may be stable or metabolically labile (see Y. Arano et al, Bioconjug. Chem. 71-76 (1991) and L. Yuanfang et al, Pure & Appl. Chem. 63, 427-463 (1991)). A labile linker may e.g. contain a hydrolysable ester group.

The chelating compounds according to the invention are suitable for complexing trivalent metal ions, in particular heavy metal ions, such as Y, Ru, Rh, In, La, the lanthanides, Bi, Ac and the actinides. For therapeutic and diagnostic purposes, radioisotopes of such metals, e.g. ^{90}Y, ^{99m}Tc, ^{111}In, ^{152}Eu, ^{153}Sm, ^{211}Bi, ^{212}Bi, ^{213}Bi or ^{225}Ac are preferably used. The invention also concerns the chelates of the chelating compounds described above with such metal ions.

Coupling of the chelating compounds and chelates described above, which have a functional link A^6-X, with targeting agents can be performed in a way known per se, e.g. by reaction of the functional group X with an amino group, hydroxy group or mercapto group of a targeting protein, by reaction of functional group X with a hydroxy group of a targeting polysaccharide (as such or a glycosyl side chain of a protein) or by reaction of functional group X with an amino group or hydroxy group of a targeting poly- or oligo-nucleotide. Methods of coupling chelators to targeting agents have been reviewed by L. Yuanfang and W. Chuanchu in *Pure & Appl. Chem.* 63(3), 427-463 (1991). The targeting agents are site-specific or receptor-specific. Preferred targeting agents are antibodies or fragments thereof, site-specific proteins, hormones or other receptor ligands and poly- or oligonucleotides.

Coupling may be achieved directly or through the intermediacy of further coupling agents such as dialdehydes or dithiols as is known in the art. Coupling of the chelating compound to the targeting molecule may be performed before or after the complexation with the metal ion in the chelating compound. Coupling can be accomplished e.g. by incubating the targeting protein and the chelating agent in a buffer and purifying the protein-chelate conjugate by gel filtration chromatography. The invention also relates to the conjugates obtained by coupling of a chelating compound or a chelate as described above.

The conjugates are useful in cases where tightly bound metal ions are required on specific sites, such as in therapy, *in vitro* diagnostics, as MRI contrast agents, etc.

The invention furthermore relates to pharmaceutical compositions containing a chelating compound, a chelate or a conjugate as described above, together with a pharmacologically acceptable carrier, such as an injection fluid or an excipient, possibly with additives, adjuvants etc.

The chelating compounds may be prepared by methods known per se. For example, the compounds of the DOTA-type and the cyclotridecane and cyclotetradecane homologues can be preprared starting for the appropriate N-unsubtituted tetraaza ring system, to which first the group $-CR^4R^8-CR^9R^{10}-Z^8-A^5-Z^9$ is attached, whereafter the three groups $-CR^1R^5-Z^5$, $-CR^2R^6-Z^6$ and $-CR^3R^7-Z^7$ are introduced. Especially when the latter three groups are identical, e.g. carboxy-methyl, it may be advantageous to start with the appropriate tri-substituted tetraaza system, such as triprotected DO3A, to which the side chain $-CR^4R^8-CR^9R^{10}-Z^8-A^5-Z^9$ is then attached.

The compounds of the calix[4]arene type may start with the appropriate tetrabenzo[*ab,ef,ij,mn*]cyclohexadecatetraene-2,6,10,14-tetraol. To the tetraol, which is taken as an example, the groups $-CR^1R^5-Z^5$, $-CR^2R^6-Z^6$, $-CR^3R^7-Z^7$, and $-CR^4R^8-CR^9R^{10}-Z^8-A^5-Z^9$ or a precursor of the latter such as $-CR^4R^8-CR^9R^{10}-Z^8Y$ (wherein Y may e.g. be hydrogen, alkyl or a protecting group), may than be attached, either simultaneously, e.g. when they are all carboxymethyl,or consecutively if they are different. Preferably Z^5, Z^6, and Z^7 are attached in a protected form. The group $-CR^4R^8-CR^9R^{10}-Z^8-A^5-Z^9$ is then introduced as such or by coupling of A^5-Z^9 (optionally protected) to Z^8 (deprotected if necessary). Finally the protecting groups are removed. Examples of these approaches are given here below.

The synthesis of 9-coordinating macrocycles fitted with a handle A^6-X for conjugation can be carried out via several routes, depending on

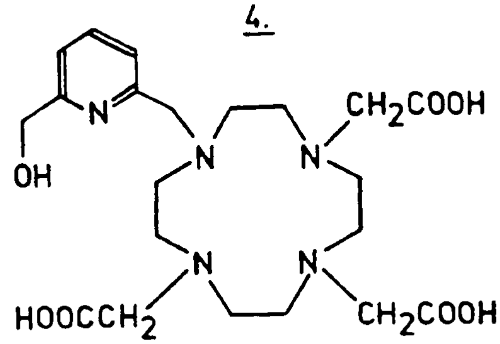
the attachment position of the handle. Examples are given below.

Synthesis of 9-coordinating tetra-aza macrocycles

Example I

Synthesis of 1-(6-hydroxymethyl-2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (compound with formula 4)

4.



2-Bromomethyl-6-hydroxymethylpyridine

2,6-Bis-hydroxymethyl-pyridine (10.0 gram, 71.8 mmol) was dissolved in 100 mL 48% HBr in H_2O. The mixture was heated at bath temperature 140°C for 2 hours, and subsequently cooled to -5°C. The solution was neutralized by dropwise addition of 40% aqueous NaOH at -5°C until pH=7. The resulting slurry was extracted with CH_2Cl_2 (5 x 100 mL). The organic layers were combined, dried over Na_2SO_4, and the solvent was evaporated to give an oil. The crude products was separated on silica. Upon elution with CH_2Cl_2 0.40 gram of bis(bromomethyl)pyridine was obtained. Further elution with ether gave 4.50 gram of 2-bromomethyl-6-hydroxymethylpyridine as a white crystalline product. Yield 31%; ^{1}H-NMR ($CDCl_3/CD_3OD$) δ: 7.75 (t, J=7Hz, 1H, PyrH), 7.38 (d, J=7Hz, 2H, PyrH), 4.72 (s, 2H, CH2Br), 4.53 (s, 2H, CH2OH).

2-Bromomethyl-6-tetrahydropyranyloxymethyl-pyridine

To a solution of 2.00 gram (9.90 mmol) of 2-bromomethyl-6-hydroxymethylpyridine in 10 ml CH_2Cl_2 was added 0.14 gram (1.98 mmol) p-toluenesulphonic acid and 3.34 gram (40 mmol) of dihydropyran. The mixture was stirred for 3 days. The reaction mixture was washed with 1% aqueous $NaHCO_3$ and dried over Na_2SO_4. The solvent was evaporated, and the excess of dihydropyran was removed by distillation under reduced pressure.

The residue was then purified by chromatography on silica with toluene/-EtOH 9/1 as the eluent to give 1.20 gram of the 2-bromomethyl-6-hydroxy-methyl-pyridine tetrahydropyranyl (THP) ether. Yield 43%. ^{1}H NMR ($CDCl_3$) δ: 7.72 (t, J=7Hz, 1H, PyrH), 7.41 (d, J=7Hz, 1H, PyrH), 7.32 (d, J=7Hz, 1H, PyrH), 4.90, (d, 1H, PyrCH) 4.62 (d, 1H, PyrCH) 4.77 (t, J=1Hz, 1H, CH-O), 4.55 (s, 2H, CH2Br), 3.72-3.98 and 3.47-3.62 (m, 2H, CH_2), 1.45-2.00 (m, 6H, 3 x CH2), MS: M+ 285 (calcd: $C_{12}H_{16}NO_2Br$ 285.036)

Tri-t-butyl 1-(6-tetrahydropyranyloxymethyl-2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetate (protected macrocycle)

To a solution of 0.5 gram (0.97 mmol) of 1,4,7-tricarboxymethyl-1,4,7,10-tetraazacyclododecane tris-t-butyl ester in 10 mL CH_3CN was added 0.60 gram (0.85 mmol) Cs_2CO_3 and 0.257 gram (0.96 mmol) 2-bromomethyl-6-tetrahydropyranyloxymethyl-pyridine. The mixture was refluxed overnight and subsequently cooled. The salts were removed by filtration and the solvent was evaporated to give the product as a white powder. ^{1}H NMR ($CDCl_3$) δ: 7.65 (t, J= 7Hz, 1H, PyrH), 7.34 (d, J=7Hz, 1H, PyrH), 7.36 (d, J=7Hz, 1H, PyrH), 4.88 (d, J=14Hz, 1H, OCH), 4.60 (d, J=14Hz, 1H, OCH), 4.78 (t, J=1Hz, 1H, CH-O), 3.99-3.85 and 3.62-3.50 (m, 2H, CH_2), 3.75 (s, CH_2), 3.35 (s, 2H, CH_2CO_2), 3.22 (s, 4H, 2 x CH_2CO_2), 2.85 (bs, 12H, CH_2N), 2.75-2.65 (m, 4H, CH_2N), 2.0-1.5 (m, 6H, 3 x CH_2 THP), 1.45 (s, 9H, tBu), 1.43 (s, 18H, tBu).

Synthesis of chelating macrocycle 4

A solution of 0.20 gram (0.28 mmol) of tri-t-butyl 1-(6-tetrahydro-pyranyloxymethyl-2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetate in 50 mL EtOAc containing an excess of HCl was stirred at room temperature for 1 hour. After addition of 0.5 mL of water the solvent was evaporated and the residue was triturated with EtOAc. The resulting solid product was filtered off to give 0.15 gram (98%) of the product as the dihydrochloride salt as a white powder. The product was recrystallised from MeOH/acetone. ^{1}H NMR (D_2O) δ 8.55 (t, J=7Hz, 1H, PyrH), 8.05 (d, J=7Hz, 1H, PyrH), 8.00 (d, J=7Hz, 1H, PyrH), 5.07 (s, 2H, CH_2OH), 4.2-3.0 (m, 24H, NCH_2). MS (FAB) M+H 468 (Calculated $C_{21}H_{34}N_5O_7$= 467).

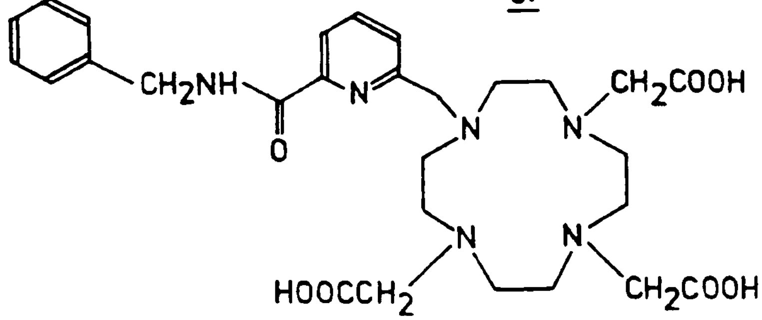
Example II

Synthesis of 1-(6-benzylcarbamoyl-2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (compound with formula 5)

2-Methoxycarbonyl-6-benzylaminocarbonyl-pyridine

To a solution of 19.5 gram (0.1 mol) 2,6-pyridinedicarboxylic acid dimethyl ester in 100 mL MeOH was added 10.7 gram (0.1 mol) of benzylamine. The solution was refluxed for 60 h. The solvent was evaporated, and the resulting product was purified by chromatography (silica, EtOAc/heptane 2/3). The yield of the monoamide monoester was 11 gram (41%). ^{1}H NMR ($CDCl_3$) δ 8.45 (d, J=7Hz, 1H, PyrH), 8.23 (d, J=7Hz, 1H, PyrH), 8.00 (t, J=7Hz, 1H, PyrH), 7.4-7.2 (m, 5H, ArH), 4.73 (d, J=5Hz, 2H, CH_2N), 3.98 (s, 3H, OCH_3). MS 270 (M+, Calculated: $C_{15}H_{14}N_2O_3$: 270)

5.



2-Hydroxymethyl-6-benzylaminocarbonyl-pyridine

To a solution of 10.0 gram (37 mmol) of 2-methoxycarbonyl-6-benzylaminocarbonylpyridine in 100 mL MeOH at 30°C was added 2.85 gram (75 mmol) $NaBH_4$ in small portions over a period of 2 hours. The mixture was stirred overnight. Water was added (50mL) and the mixture was concentrated to 25 mL. Another portion of 100 mL water was added and the mixture was extracted with 3x100 mL of CH_2Cl_2. The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated to give an oil which solidified upon standing. Yield 8.5 gram (95%), Mp 89.5-92.0°C, ^{1}H NMR ($CDCl_3$) δ 8.4-8.2 (bs, 1H, NH), 8.15 (d, J=7Hz, 1H, PyrH), 7.85 (t, J=7Hz, 1H, PyrH), 7.48 (d, J=7Hz, 1H, PyrH), 7.4-7.2 (m, 5H, ArH), 4.78 (d, J=5Hz, 2H, CH_2O), 4.68 (d, J=6Hz, 2H, CH_2N), 3.16 (bt, 1H, OH). MS 242 (M+, calcd $C_{14}H_{14}N_2O_2$: 242)

2-Chloromethyl-6-benzylaminocarbonyl-pyridine

A solution of 8.5 gram (35 mmol) of 2-hydroxymethyl-6-benzylamino-carbonylpyridine in 150 mL CH_2Cl_2 was treated with 6.75 gram (35 mmol) tosyl chloride and 7.07 gram (70 mmol) Et_3N at room temperature. The mixture was stirred overnight. The solution was then washed with H2O, and dried over MgSO4. After evaporation of the solvent, the product was purified by flash chromatography (silica, EtOAc/heptane 1/1), to give the product as an oil, which solidified upon standing. Yield 3.0 gram. ^1H NMR ($CDCl_3$) δ 8.3 (bs, 1H, NH), 8.18 (d, J=7Hz, 1H, PyrH), 7.89 (t, J=7Hz, 1H, PyrH), 7.6 (d, J=7Hz, 1H, PyrH), 7.4-7.2 (m, 5H, ArH), 4.67 (d, J=5Hz, 2H, CH_2N), 4.65 (s, 2H, CH_2Cl).

Tri-t-butyl1-(6-benzylaminocarbonyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetate (protected macrocycle)

To a solution of 0.23 gram (0.45 mmol) 1,4,7-tricarboxymethyl-1,4,7,10-tetraazacyclododecane tris-t-butyl ester in 10 mL EtOH was added 0.12 gram 2-chloromethyl-6-benzylaminocarbonylpyridine and 0.25 gram (2.35 mmol) Na_2CO_3. After addition of 5 mg NaI the mixture was refluxed for 70 hours. The solvent was evaporated and the product was dissolved in CH_2Cl_2/H_2O and this was extracted with CH_2Cl_2. The organic layers were combined and dried over Na_2SO_4. After evaporation of the solvent, the product was purified by chromatography to give 90 mg of product. ^1H NMR ($CDCl_3$) δ 8.95 (bt, 1H, NH), 8.25 (d, J=7Hz, 1H, PyrH), 7.90 (t, J=7Hz, 1H, PyrH), 7.35-7.20 (m, 5H, ArH), 4.7 (bs, 2H, NCH_2), 3.7 (m, 2H, $PyrCH_2$), 3.3-2.0 (bm, 24H, NCH_2), 1.5 (s, 9H, tBu), 1.35 (s, 18H, 2x tBu).

Synthesis of chelating macrocycle 5

A solution of 80 mg (0.1 mmol) of tri-t-butyl 1-(6-benzylaminocarbonyl-2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetate in 50 mL CH_2Cl_2 was saturated with HCl gas and the mixture was stirred overnight at room temperature. The solvent was evaporated and the product was dissolved in 0.5 mL of MeOH. Upon addition of EtOAc the product precipitated and this was filtered off, to give a white powder. Yield: 65 mg (100%). ^1H NMR (CD_3OD) δ 8.05-7.75 (m, 3H, PyrH), 7.45-7.10 (m, 5H, ArH), 4.60 (s, 2H, $ArCH_2$), 4.5-3.0 (m, 24H, NCH_2). FAB MS 571 (M+H: Calculated for $C_{28}H_{38}N_6O_7$: 570)

Example III

Synthesis of 1-(6-methoxycarbonyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid (compound with formula 6a)

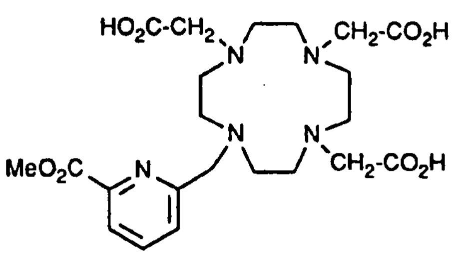
and

Synthesis of 1-(6-carboxyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid (compound with formula 6b)

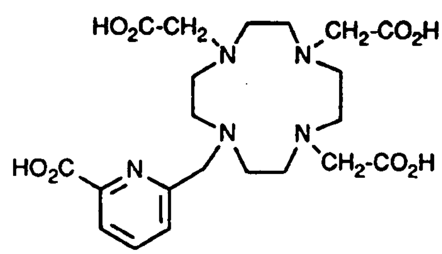
2-Hydroxymethyl-6-methoxycarbonyl-pyridine

A solution of 2,6-pyridinedicarboxylate dimethyl ester (10.0 gram, 43 mmol) in 500 mL MeOH at 40°C was treated with $NaBH_4$ (2.47 gram, 65 mmol), which was added in portions over 1 hour. The mixture was stirred overnight. Water (100 mL) was added, and the product was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4, and concentrated in vacuo. The product was purified by chromatography (silica, EtOAc/heptane 1/3). Yield: 34%. ^1H NMR ($CDCl_3$) δ 8.15 (d, J=7Hz, 1H, PyrH, 7.87 (t, J=7Hz, 1H, PyrH), 7.55 (d, J=7Hz, 1H, PyrH), 4.88, (bs, 2H, CH_2OH), 4.00 (s, 3H, OCH_3).

6a.



6b.



2-Methanesulphonyloxymethyl-6-methoxycarbonyl-pyridine

A solution of 2-hydroxymethyl-6-methoxycarbonyl-pyridine (1.67 gram, 10 mmol) in 100 mL CH_2Cl_2 was cooled to 0°C. Et_3N (2.02 gram, 20 mmol) was added, followed by methanesulfonyl chloride (1.35 gram, 11 mmol). The mixture was stirred for 1 hour, diluted with 200 mL CH_2Cl_2, and washed with H_2O. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The product was purified by chromatography (silica, CH_2Cl_2/EtOH 98/2). Yield: 41%. ^1H NMR ($CDCl_3$) δ 8.15 (d, J=7Hz, 1H, PyrH), 7.92 (t, J=7Hz, 1H, PyrH), 7.68 (d, J=7Hz, 1H, PyrH), 5.45, (s, 2H, CH_2O), 4.00 (s, 3H, OCH_3), 3.15 (s, 3H, SCH_3).

Tri-t-Butyl 1-(6-methoxycarbonyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid (protected macrocycle)

To a solution of 2.05 gram (4.0 mmol) of tri-t-butyl-1,4,7,10-tetraaza-cyclododecane-1,4,7-triacetate in 50 mL CH_3CN was added 0.98 gram (4.0 mmol) 2-methanesulfonyloxymethyl-6-methoxy-

carbonyl-pyridine and 1.30 gram (4.0 mmol) Cs_2CO_3. The mixture was stirred at 50°C for 5 hours. After cooling to room temperature the salts were filtered off, and the organic layer was concentrated. The residue was taken up in CH_2Cl_2 and washed with H_2O. The organic layer was dried (Na_2SO_4) and concentrated. The product was purified by chromatography (silica, CH_2Cl_2/EtOH 95/5) Yield 50%. ^1H NMR ($CDCl_3$) δ 8.04 (d, J=7Hz, 1H, PyrH), 7.95 (t, J=7Hz, 1H, PyrH), 7.58 (d, J=7Hz, 1H, PyrH), 3.92 (s, 3H, OCH_3), 3.4-2.2 (m, 24H, NCH_2), 1.50 (s, 9H, tBu), 1.32 (s, 18H, 2 x tBu). FAB MS: 664 $(M+H)^+$, 686 $(M+Na)^+$ (Calcd for $C_{34}H_{57}N_5O_8$: 663).

Synthesis of chelating macrocycle 6a

A solution of 1.0 gram of tri-t-butyl 1-(6-methoxycarbonyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid in 100 mL CH_2Cl_2 was saturated with HCl gas, and the solution was kept overnight. The mixture was concentrated to 10 mL. The product precipitated and was filtered off, and was washed with CH_2Cl_2, EtOAc, and ether, and was dried in vacuo. Yield 70%. ^1H NMR (D_2O) δ 8.20 (d, J=7Hz, 1H, PyrH), 8.05 (t, J=7Hz, 1H, PyrH), 7.95 (d, J=7Hz, 1H, PyrH), 4.00 (s, 3H, OCH_3) 3.9-2.9 (m, 24H, NCH_2). FAB MS: 496 $(M+H)^+$ (Calculated for $C_{22}H_{33}N_5O_8$: 495).

Synthesis of chelating macrocycle 6b

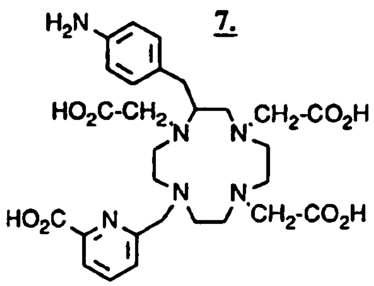
A solution of compound 6a (0.25 gram, 0.5 mmol) in 10 mL dioxane/H_2O 7/3 was treated with excess LiOH (pH=11) at room temperature overnight. After evaporation of dioxane, the product was acidified with HCl to pH=1, and precipitated with AtOH/ether. FAB-MS: 482 $(M+H)^+$ (Calculated for $C_{21}H_{31}N_5O_8$: 481).

Example IV

Synthesis of 1-(6-carboxyl-2-pyridylmethyl)-5-(4-aminobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid (compound with formula 7)

2-Aminobenzyl-3-oxo-1,4,7-triaza-heptane

A solution of 8.07 gram (36 mmol) DL-p-nitro-phenylalanine methyl ester was dissolved in 10 mL MeOH, and added dropwise to 75 mL ethylene diamine at room temperature. The mixture was stirred overnight at room temperature. The solvents were removed in vacuo, and the residue solidified upon stirring with EtOAc. The product was filtered off. Yield: 99%. ^{1}H NMR (D_2O) 8.15 (d, 2H, ArH), 7.35 (d, 2H, ArH), 3.62 (dd, 1H, αH), 3.4-2.7 (m, 5H, CH_2NH, CHNH, $ArCH_2$), 2.40-2.65 (m, 2H, CH_2NH).



7.

N-(t-Butoxycarbonyl)-iminodiacetic acid

A suspension of 20 gram (0.15 mol) iminodiacetic acid in 500 mL $tBuOH/H_2O$ (2/1) was treated with 4N NaOH until pH 8.5. The starting material dissolved. Boc_2O (36 gram ,0.16 mol) was dissolved in $tBuOH/H_2O$ (2/1) and this was added rapidly throough a dropping funnel, while keeping the pH at 8 by addition of 4N NaOH. The mixture was stirred overnight at room temperature. The mixture was diluted with 200 mL water, and was washed with heptane. The pH was adjusted to pH=3 with $KHSO_4$, and the product was isolated by extensive extraction with EtOAc. The organic layers were combined, and dried over Na_2SO_4, and the solvent was evaporated to give an oil. Yield 72%. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H, tBu), 4.00 (s, 2H, NCH_2), 4.10 (s, 2H, NCH_2), 4.5 (bs, 2H, COOH).

N-(t-Butoxycarbonyl)-iminodiacetic acid bis N-hydroxysuccinimide ester.

A solution of 15 gram (64 mmol) N-(t-butoxycarbonyl)-iminodiacetic acid in 1500 mL CH_2Cl_2 was cooled to 0°C. 26.6 gram (128 mmol) dicyclohexyl-carbodiimide and 14.8 gram (128 mmol) N-hydroxysuccinimide were added, and the mixture was stirred at room temperature overnight. The precipitated dicyclohexylurea was removed by filtration. The solvent was evaporated to give 26.7 gram (97%) of the product. ^{1}H NMR ($CDCl_3/CD_3OD$)) δ 1.50 (s, 9H, tBu), 2.86 (s, 4H, CH_2CH_2), 2.89 (s, 4H, CH_2CH_2), 4.45 (s, 2H, NCH_2), 4.58 (s, 2H, NCH_2).

1-(t-Butoxycarbonyl)-5-(4-nitrobenzyl)-3,6,11-trioxo-1,4,7,10-tetraaza-cyclododecane.
A solution of 2-aminobenzyl-3-oxo-1,4,7-triaza-heptane (9.3 gram, 37 mmol) and 6 mL Et_3N in 200 mL of dry DMF/dioxane (1/1) and a solution of 15.8 gram of N-(t-butoxycarbonyl)-iminodiacetic acid bis N-hydroxysuccinimide ester in 200 mL of dry dioxane were added dropwise over 1 hour to a solution of 500 mL of refluxing dioxane. The mixture was refluxed overnight. After cooling to room temperature the solvent was evaporated. Acetone was added and the product precipitated, and was filtered off. Yield: 10%. ^{1}H NMR (DMSO-d_6) δ 8.65 (bd, 1H, NH), 8.18 (d, 2H, ArH), 7.75 (bt, 1H, NH), 7.55 (d, 2H, ArH), 7.15 (b, 1H, NH). 4.4 (bq, 1H, αH), 4.2-2.9 (m, 8H, 4 x CH_2), 1.45 (s, 9H, tBu). FAB-MS 450 $(M+H)^+$ (calcd for $C_{20}H_{27}O_7N_5$: 449).

1-(t-Butoxycarbonyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane.
A solution of 1.0 gram (2.2 mmol) of 1-(t-butoxycarbonyl)-5-(4-nitrobenzyl)-3,6,11-trioxo-1,4,7,10-tetraaza-cyclododecane in 20 mL of dry THF under N_2 was cooled to -15°C . A solution of BH_3.THF (1 M, 30 mL) was added and the mixture was stirred at -15°C for 1 hour and subsequently heated to reflux for 18 hours. After cooling to room temperature 10 mL of MeOH was added carefully, and the solvent was evaporated. The residue was taken up in EtOAc and the product was precipitated with ether. Yield 56%. NMR (DMSO-d_6) δ 8.1 (bd, 2H, ArH), 7.5 (bd, 2H, ArH), 7.5 (bm, 1H, NH), 6.7 (bm, 1H, NH), 6.4 (bm, 1H, NH), 4.9 (bm, 1H, αH), 3.7-2.1 (m, 16 H, CH_2), 1.4 (s, 9H, tBu). FAB-MS 408 $(M+H)^+$ (calcd for $C_{20}H_{33}N_5O_4$: 407).

1-(t-Butoxycarbonyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid triethyl ester.
To a solution of 0.15 gram (0.36 mmol) of 1-(t-butoxycarbonyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane in 20 mL CH_3CN was added 0.50 gram (1.53 mmol) Cs_2CO_3 and the mixture was heated to reflux temperature. Subsequently 0.4 gram (2.39 mmol) of ethyl bromoacetate was added. The mixture was refluxed for 1 hour. The solvent was evaporated. The residue was dissolved in H_2O/CH_2Cl_2. The organic layer was separated off, dried over Na_2SO_4, and the solvent was evaporated. Yield: 62%. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, 2H, ArH), 7.32 (d, 2H, ArH), 4.3-4.1 (m, 6H, 3 x OCH_2), 4.1-4.0 (m, 1H, αH), 3.9-2.4 (m, 22H, 11 x CH_2), 1.48 (s, 9H, tBu), 1.35-1.05 (m, 9H, 3 x CH_3). FAB-MS 666 $(M+H)^+$, calcd for $C_{32}H_{51}N_5O_{10}$ 665.

2-(4-Nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-triacetic acid triethyl ester.
A solution of 0.14 gram (0.21 mmol) of 1-(t-butoxycarbonyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid triethyl ester in 50 mL CH_2Cl_2 was treated with HCl gas for 30 minutes. The mixture was stirred at room temperature for 1 hour. The solvent was evaporated and the residue was dissolved in H_2O, and extracted with CH_2Cl_2. The organic layer was dried on Na_2SO_4 and the solvent was evaporated. FAB-MS: 566 $(M+H)^+$ (Calcd for $C_{27}H_{43}N_5O_8$: 565)

1-(6-Methoxycarbonyl-2-pyridylmethyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid triethyl ester

To a solution of 0.11 gram (0.19 mmol) of 2-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-triacetic acid triethyl ester in 20 mL CH_3CN was added 0.25 gram (0.95 mmol) Cs_2CO_3 The mixture was heated to reflux for 2 hours, and 0.048 gram (0.19 mmol) of 2-methanesulphonyloxymethyl-6-methoxycarbonyl-pyridine was added. The mixture was heated for an additional 2 hours. After cooling to room temperature the salts were removed by filtration. The filtrate was concentrated and dissolved in EtOAc, washed with water, and dried over Na_2SO_4. Yield: 91%. ^1H NMR ($CDCl_3$) δ 8.15 (d, 2H, ArH), 8.10-7.85 (m, 2H, PyrH), 7.80-7.65 (m, 1H, PyrH),7.20 (d, 2H, ArH), 4.3-4.05 (m, 7H, 3 x OCH_2 + αH), 3.9-2.2 (m, 24H, CH_2N), 1.4-1.1 (m, 9H, 3 x CH_3).

1-(Carboxyl-2-pyridylmethyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid

To a solution of 0.15 gram (0.17 mmol) of 1-(6-methoxycarbonyl-2-pyridylmethyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid triethyl ester in 20 mL of dioxane/H_2O (7/3) was added LiOH.H_2O to pH=11. The mixture was stirred a t room temperature overnight. 2M HCl was added to pH=1, and the product was precipitated with EtOH/ether. ^1H NMR (D_2O) δ 8.40 (d, 2H, ArH), 8.35-8.1 (m, 2H, PyrH), 7.50 (d, 1H, PyrH), 7.50 (d, 2H, ArH), 4.3-2.6 (m, 25H, CH_2, CH). FAB-MS: 617 (Calcd for $C_{28}H_{36}N_6O_{10}$: 616).

Synthesis of chelating macrocycle 7

A solution of 21.6 mg (0.07 mmol) of $SnCl_2$ in 5 mL of concentrated HCl was heated to 110°C. To this solution 30 mg (0.05 mmol) of 1-(carboxyl-2-pyridylmethyl)-5-(4-nitrobenzyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid in 5 mL HCl was added and heating was continued for 2 hrs. After cooling to room temperature, the solvent was evaporated and the product was purified by HPLC (fosfate buffer/water/acetonitrile). The product was desalted by the HPLC with HCl/water/acetonitrile. Yield: 16%. ^1H NMR (D_2O) δ 8.1-7.9 (m, 3H, PyrH), 7.4-6.9 (m, 4H, ArH), 4.0-2.6 (m, 25H, CH, CH_2). FAB-MS 587 $(M+H)^+$ (Calcd for $C_{28}H_{38}N_6O_8$ 586).

Example V

a) Results of complexation experiments of 1-(6-hydroxymethyl-2-pyridylmethyl)-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (macrocycle 4)

$^{225}Ac^{3+}$ complexation

temperature	25°C	50°C	70°C
% $^{225}Ac^{3+}$ complex	71%	78%	86%

$^{152}Eu^{3+}$ complexation

temperature	25°C	50°C	70°C
% $^{152}Eu^{3+}$ complex	93%	90%	100%

$^{241}Am^{3+}$ complexation

temperature	25°C	50°C	70°C
% $^{241}Am^{3+}$ complex	82%	80%	89%

Competition experiment with Eu^{3+}, starting with the ^{225}Ac complex; Excess Eu^{3+}, 10x relatieve to chelator.

temperature	25°C	50°C	70°C
% $^{225}Ac^{3+}$ complex	83%	83%	89%.

Comparative example

Synthesis of tetra-p-t-butyl-tetrabenzo[ab,ef,ij,mn]cyclohexadecatetraene-2,6,10,14-tetra(oxyacetic acid) mono(propylamide) (calix[4]arene-tetraoxyacetic acid mono(propylamide)) (compound with formula 8, R = H)

Calix[4]arene-tetraoxyacetic acid triethyl ester monopropylamide (compound with formula 8, R = ethyl).

Monoacid calix[4]arene-tetraoxyacetic acid triethyl ester with formula 9 (Bohmer, V. et al. *J. Chem. Soc., Perkin Trans. 1.* 1990. 431.) (1.5 g, 1.56 mmol) was refluxed in $SOCl_2$ (10 mL) for 3 hours. The solvent was evaporated in vacuo, and residual solid was dissolved in CH_2Cl_2 (10 mL). N-Propylamine (0.92 g, 15.6 mmol) in CH_2Cl_2 (10 mL) was then added. The reaction mixture was stirred overnight at room temperature, evaporated, dissolved in CH_2Cl_2 (10mL), washed with H_2O, dried ($MgSO_4$), evaporated and recrystallized from EtOH-H_2O, 4:1. Yield 77%; ^1H NMR ($CDCl_3$) δ 8.46 (br s, 1H), 6.89 (s, 2H) 6.81 (s, 4H), 6.70 (s, 2H), 5.0-4.5 (m, 16H), 4.15 (q, J = 7.0 Hz, 6H), 3.30 (m, 2H), 1.68 (m, 2H), 1.10 (s, 9H), 1.04 (s, 18H), 1.00 (s, 9H), 0.97 (t, J = 7.0 Hz, 3H). Mass spectrum (FAB), m/z 1006.8 ($(M+H)^+$, calcd 1006.6).

Example V

b)

Results of complexation experiments of 1-(6-methoxycarbonyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid (macrocycle 6a)

$^{225}Ac^{3+}$complexation

Temperature	25 °C	50 °C	70 °C
% $^{225}Ac^{3+}$complex	8%	79%	100%

Competition experiment with Eu^{3+}, starting with the $^{225}Ac^{3+}$ complex;
Excess Eu^{3+}, 10x relative to chelator.

Temperature	25 °C	50 °C	70 °C
% $^{225}Ac^{3+}$complex	96%	97%	61%

c)

Results of complexation experiments of 1-(6-carboxyl-2-pyridylmethyl)-1,4,7,10-tetraaza-cyclododecane-4,7,10-triacetic acid (macrocycle 6b)

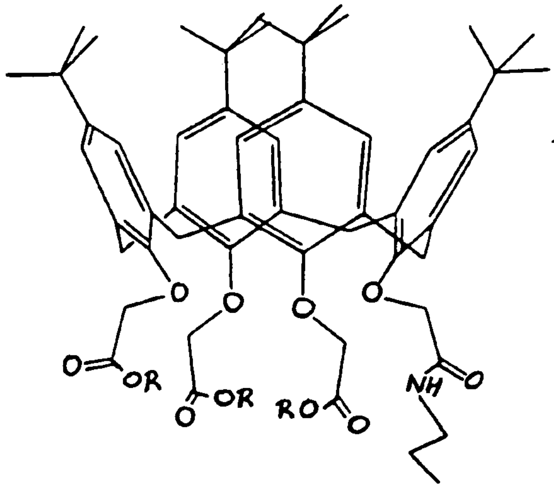
$^{225}Ac^{3+}$complexation

Temperature	25 °C	50 °C	70 °C
% $^{225}Ac^{3+}$complex	20%	92%	100%

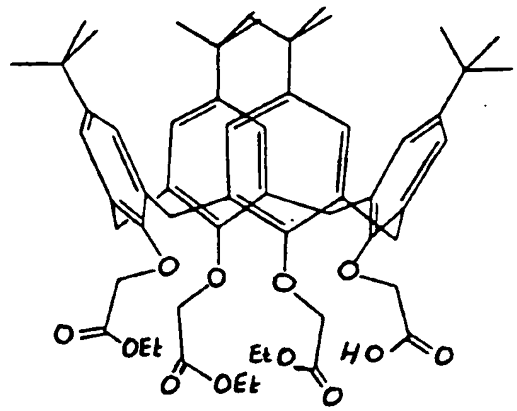
Competition experiment with Eu^{3+}, starting with the $^{225}Ac^{3+}$ complex;
Excess Eu^{3+}, 10x relative to chelator.

Temperature	25 °C	50 °C	70 °C
% $^{225}Ac^{3+}$complex	96%	91%	38%

8.



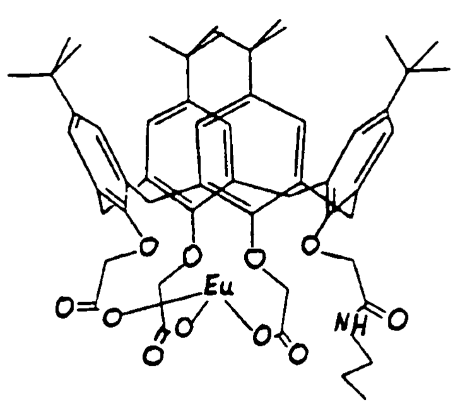
9.



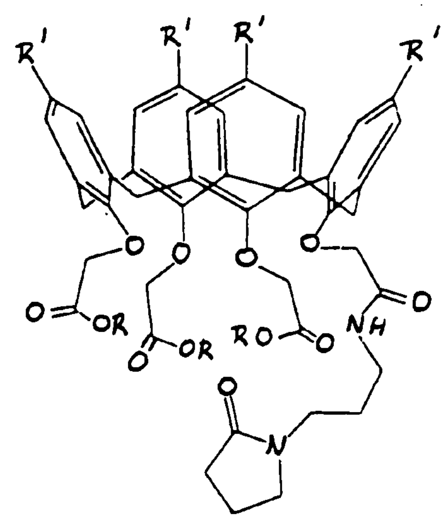
Calix[4]arene-tetraoxyacetic acid monopropylamide (compound with formula 8, R = H).

The triester monoamide (0.5 g, 0.49 mmol) and K_2CO_3 (1.38 g, 10 mmol) were refluxed in $MeOH-H_2O$ (20 mL, 5:1) for 1 hour, acidified (pH 4). The product was extracted with EtOAc (2 x 25 mL), dried ($MgSO_4$), the solvent was evaporated. Yield 54%; ^1H NMR ($CDCl_3$) δ 7.19 (s, 4H) 6.76 (s, 2H), 6.38 (s, 2H), 5.0-3.0 (m, 18H), 1.85 (m, 2H), 1.41 (s, 18H), 0.97 (t, J = 7.0 Hz, 3H), 0.95 (s, 9H), 0.81 (s, 9H).

10.



11.



Complex of 8 with Eu^{3+} (formula 10).

$EuCl_3.6H_2O$ (0.032 g, 0.09 mmol) and trimethyl orthoformate (excess) were refluxed in dried MeCN (10 mL) for 1 hour. The triacid with formula 8 (R = H) (0.09 mmol) and equimolar Et_3N in MeOH (5 mL) were added, and the mixture was refluxed for 3 hours, evaporated, washed with H_2O (2x15 mL) and dried. Mass spectrum (FAB), m/z 1070.9 (M+H)., calcd 1070.6). Anal. Calcd. for $C_{55}H_{71}NO_{11}Eu$:0.5$CHCl_3$ (M_r 1169.6): C, 58.54; H, 6.32; N, 1.23. Found: C,58.89;H,6.62;N,1.20.

Example VI

Synthesis of calix[4]arene compounds (compounds with formula 11, R' = t-butyl).

Triester mono(3-(2-oxo-1-pyrrolidinyl)propyl)amide (compound with formula 11, R = Et, R' = t-Bu).

This amide was obtained in a way analogous to the procedure for the mono-amide with formula 8 (R = Et), starting with monoacid of formula 9. ^1H NMR ($CDCl_3$) δ 8.50 (br s, 1H), 6.89 (s, 6H), 6.71 (s, 2H), 5.0-1.8 (m, 34H), 1.31 (t,J = 7.0 Hz, 9H), 1.11 (s, 9H), 1.09 (s, 18H), 0.96 (s, 9H). Mass spectrum (FAB), m/z 1089.7 ($(M+H)^+$, calcd 1088.6).

Triacid mono(3-(2-oxo-1-pyrrolidinyl)propyl)amide (compound with formula 11, R = H, R' = t-Bu)λ

This was obtained analogous to the procedure for the triacid with formula 8 (R = H). Yield 93%. ^1H NMR ($CDCl_3$) δ 7.90 (br s, 1H), 7.11 (s, 4H, Ar H), 6.83 (s, 2H), 6.51 (s, 2H), 6.0 (br s, 3H), 5.0-3.0 (m, 22H), 2.41 (t,J - 7.0 Hz, 2H), 2.00 (m, 4H), 1.37 (s, 18H, 1.00 (s, 9H), 0.91 (s, 9H). Mass spectrum (FAB), m/z 1005.3 ($(M+H)^+$, calcd 1005.6. Anal. Calcd. for $C_{65}H_{76}N_2O_{12}$ (M_r 1005.2): C, 72.47; H, 7.11: N,2.60. Found: C, 72.61; H, 7.41: N, 2.51.

Complex with Eu^{3+} (formula 12, R' = t-Bu).

This was obtained analogous to the procedure for the complex with formula 10. Mass spectrum (FAB), m/z 1155.5 ($(M+H)^+$, calcd 1155.6). Anal. Calcd. for $C_{59}H_{76}N_2O_{12}Eu.CH_2Cl_2$ (M_r 1154.6):C, 57.12; H, 6.27; N,2.25. Found: C, 57.30; H, 6.46; N, 2.36.

Example VII

Synthesis of calix[4]arene compounds (compounds with formula 11, R' = hydrogen).

Triester mono(3-(2-oxo-1-pyrrolidinyl)propyl)amide (compound with formula 11, R = Et, R' = H).

This was obtained in a way analogous to example VI. Mass spectrum (FAB), m/z 865.4 ($(M+H)^+$, calcd 865.4).

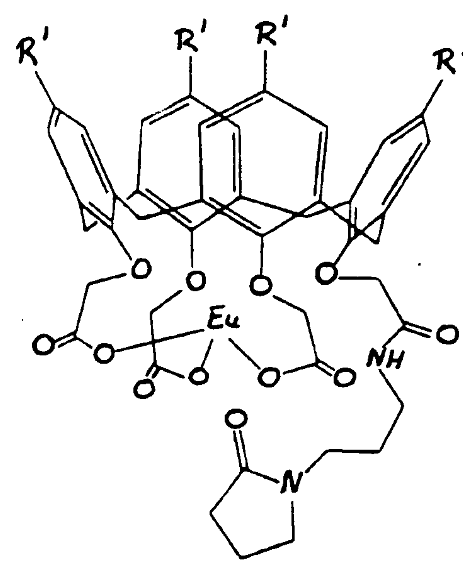
Triacid mono(3-(2-oxo-1-pyrrolidinyl)propyl)amide (compound with formula 11, R = R' = H).

Triester (formula 11, R = Et, R' = H) (0.83 g, 0.96 mmol) and K_2CO_3 (1.32 g, 9.6 mmol) were refluxed in MeOH-H_2O (5:1, 12 mL) for 1 hour, acidified (pH 4). The product was extracted with CH_2Cl_2 (2 x 25 mL), dried ($MgSO_4$), the solvent was evaporated. The product was recrystallized from EtOH-H_2O, 4:1. Mass spectrum (FAB), m/z 781.4 $(M+H)^+$, calcd (781.3). Anal. Calcd. for $C_{43}H_{44}N_2O_{12}\cdot 0.5CH_2Cl_2$ (M_r 780.3): C: 62.80; H: 5.39; N: 3.40. Found: C: 63.13; H: 5.67; N: 3.71

Complex with Eu^{3+} (formula 12, R' = H).

This was obtained in the same way as in example VI. Mass spectrum (FAB), m/z 931.2 ($(M+H)^+$, calcd (931.2). Anal. Calcd. for $C_{43}H_{44}N_2O_{12}Eu$ (M_r 931.2): C, 55.46; H, 4.76; N, 3.00. Found: C, 55.32; H, 4.82; N, 2.89.

12.



Example VIII

Synthesis of calix[4]arene compounds (compounds with formula 13)

Triester mono(3-acetylamidopropyl)amide (compound of formula 13, R = Et)

This was obtained analogous to the procedure for the monoamide of the comparative example (formula 8). Yield 82%. ^{1}H NMR ($CDCl_3$) δ 8.63 (br s, 1H), 7.14 (br s, 1H), 6.84 (s, 2H) 6.76 (s, 26H), 5.0-3.0 (m, 26H), 1.97 (s, 3H), 1.25 (t,J = 7.0 Hz, 9H), 1.11 (s, 9H), 1.06 (s, 9H), 1.04 (s, 18H). Mass spectrum (FAB), m/z 1049.5 ($(M+H)^+$, calcd. 1049.0). Anal. Calcd. for $C_{62}H_{84}N_2O_{12}\bullet CH_3CN$ (M_r 1089.0): C, 69.08; H, 8.00; N, 3.00. Found: C, 68.77; H, 7.86; N, 3.35.

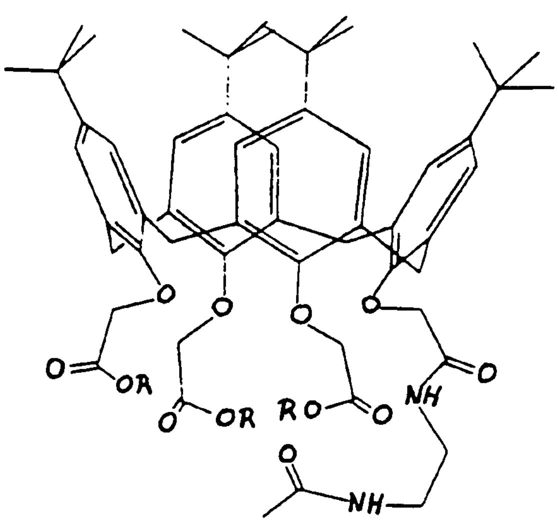
Triacid mono(3-acetylamidopropyl)amide (formula 13, R = H).

This was obtained analogous to procedure for the triacid of example VII. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 8.32 (br s, 3H), 8.01 (br s, 2H), 7.14 (s, 4H), 6.66 (s, 2H), 6.49 (s, 2H), 5.1-3.1 (m, 20H), 2.16 (s, 3H), 1.31 (s, 18H), 0.91 (s, 9H, CH_3), 0.77 (s, 9H). Mass spectrum (FAB), m/z 965.6 ($(M+H)^+$, calcd. 965.5).

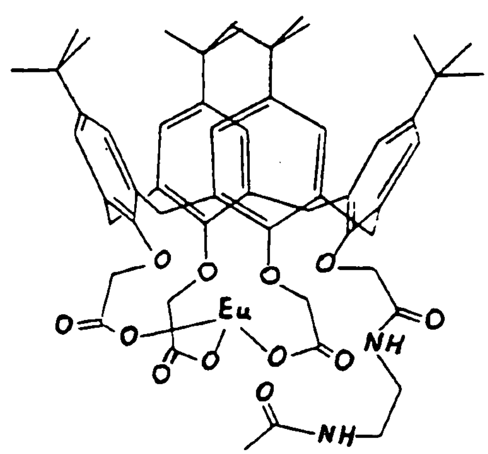
Complex with Eu^{3+} (formula 14).

This was obtained in the same way as the complex with formula 12 (example VII). Mass spectrum (FAB), m/z 1115.5 ($(M+H)^+$, calcd 1115.4).

13.



14.



Example IX

Fluorescence of the calix[4]arene triacid mono(3-(2-oxo-1-pyrrolidinyl)propyl)amide complex with Eu^{3+} (formula 12, R = t-Bu).

The fluorescence measurements of the Eu^{3+} complex with formula 12 (R = t-Bu) made in situ (dissolving the ligand with formula 11, R = H, R' = t-Bu) and $EuCl_3.6H_2O$ in MeOH and adding a small excess of Et_3N) showed a luminescence lifetime 0.900 ms (for complex 12 higher than 0.900 ms). These luminescence lifetimes are larger than reported by Ungaro et al, (J. Chem. Soc., Chem. Commun. 1990, 878) for an analogous compound. Correspondingly the luminescence quantum yield are also high with the complexes containing ninth coordination centre. The solid complexes with formula 12 (R' = H or t-Bu) also showed high luminescence upon excitation. These results show that the Eu^{3+} is efficiently shielded from solvent molecules, like MeOH, H_2O.

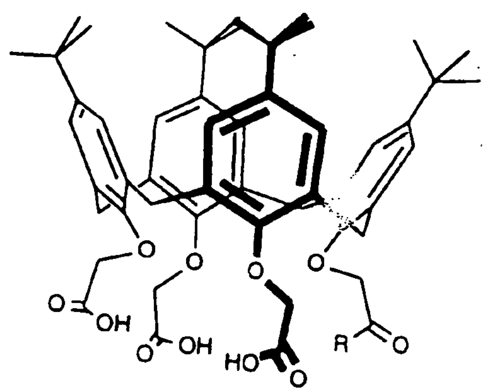
Example X

Using similar methods as described before were prepared (formula 15):

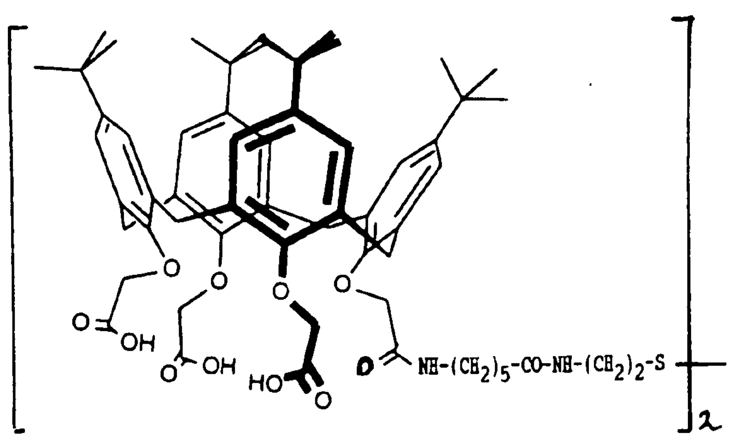
(a) R=NH-CH_2-pyridine

(b) NH-$(CH_2)_2$-pyridine

(c) NH-$(CH_2)_5$-CO-NH-$(CH_2)_2$-SH



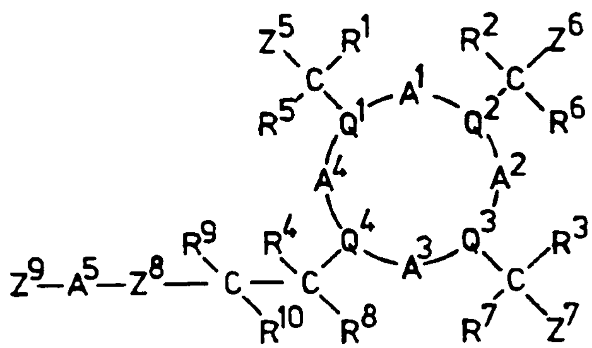
Compound 15c was converted into the compound with formula 16 using tributylphosphine:



Claims

1. Chelating compound having nine ligand sites capable of simultaneously chelating a single metal cation, the compound having formula 1:

1.



wherein:

A^1, A^2, A^3 and A^4 represent optionally substituted organic chains;

A^5 represents an optionally substituted organic chain;

Q^1, Q^2, Q^3 and Q^4 represent Z^1, Z^2, Z^3 and Z^4 respectively, in which case each of these is a nitrogen atom, or Q^1, Q^2, Q^3 and Q^4 represent C-Z^1, C-Z^2, C-Z^3 and C-Z^4 respectively, in which case Z^1, Z^2, Z^3 and Z^4 are exocyclic and independently represent O, S or NR^{11}; wherein the spatial distances between Z^1 and Z^2, Z^2 and Z^3, Z^3 and Z^4, and Z^4 and Z^1 each can be 3.15 ± 0.25 Å, and the spatial distances between Z^1 and Z^3, Z^2 and Z^4 each can be 4.45 ± 0.35 Å;

Z^5, Z^6 and Z^7 independently represent carboxyl, thiocarboxyl, carboximidoyl, phosphono, thiophosphono, phosphorimidoyl, sulpho, thiosulpho, sulfamoyl;

Z^8 represents O, S or NR^{12};

Z^9 represents O, S or NR^{13}, to which a hydrogen atom or an organic substituent may be attached;

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} independently represent hydrogen, C_1-C_6 alkyl or C_2-C_6 alk(adi)enyl, or any pair of R^1 and R^5, R^2 and R^6,

R^3 and R^7, R^4 and R^8, and R^9 and R^{10} may also represent oxo, or R^8 and R^9 or R^9 and a substituent at A^5 may be connected to form a ring together with the carbon atoms to which they are bound;

R^{11} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl or C_1-C_6 hydroxyalkyl;

R^{12} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 hydroxyalkyl or a second bond together with one of the neighbouring chain atoms of Z^8;

R^{13} represents hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 hydroxyalkyl, optionally substituted C_5-C_{10} aryl or C_6-C_{14} aralkyl, or a second bond together with the neighbouring chain atom of Z^9;

wherein one of R^1, R^2, R^3, R^4 and R^9 or one of the substituents on A^1, A^2, A^3, A^4, A^5 or Z^9 may represent A^6-X, wherein A^6 represents a direct bond or an organic linking group, and X represents a functional group capable of binding to a targeting molecule;

or a mono- or polyanion thereof derived by abstraction of one or more acidic hydrogens;

with the proviso that if Q^1, Q^2, Q^3 and Q^4 are each nitrogen and Z^9 is hydroxy, C-R^9R^{10}-Z^8 does not represent methyleneoxy or carbonylimino.

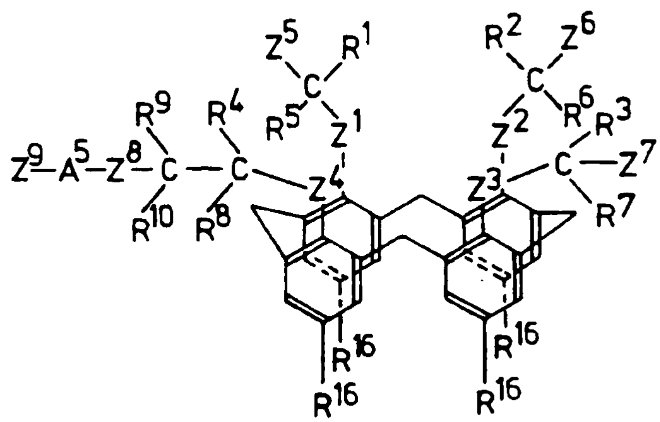
2. Chelating compound according to claim 1, wherein A^1, A^2, A^3 and A^4 each represent -CHR^{14}-CHR^{15}-, and Q^1, Q^2, Q^3 and Q^4 each represent an endocyclic nitrogen atom, wherein R^{14} and R^{15} independently represent hydrogen or C_1-C_6 alkyl, and one of the four R^{14} may represent the group A^6-X.

3. Chelating compound according to claim 2, wherein A^1, A^2, A^3 and A^4, and Q^1, Q^2, Q^3 and Q^4 together represent a 1,4,7,10-tetraaza-cyclododecane.

4. Chelating compound containing a tetrabenzo[*ab,ef,ij,mn*]cyclohexadecatetraene (calix[4]arene) group, having nine ligand sites capable of simultaneously chelating a single metal cation, said ligand sites being selected from nitrogen, oxygen and sulphur atoms, the compound optionally containing a functional group capable of binding to a targeting agent.

5. Chelating compound according to claim 1 or 4, wherein A^1, A^2, A^3 and A^4, and Q^1, Q^2, Q^3 and Q^4 together form a tetrabenzo[*ab,ef,ij,mn*]-cyclohexadecatetraene, and Z^1, Z^2, Z^3 and Z^4 represent O, attached at positions 2, 6, 10 and 13 of the cyclohexadecatetraene according to formula 2:

2.



wherein R^{16} represents hydrogen or C_1-C_6 alkyl, and one of the four R^{16} may represent the group A^6-X.

6. Chelating compound according to one of the preceding claims, wherein (Z^8)-A^5-(Z^9) represents (Z^8)-$CR^{17}R^{18}$-$(CHR^{21})_n$-$CR^{19}R^{20}$-(Z^9), wherein R^{17}, R^{18}, R^{19}, R^{20} and R^{21} independently represent hydrogen or C_1-C_6 alkyl, and R^{17} and R^{18}, or R^{19} and R^{20} may also together represent oxo, and wherein R^9 and R^{17}, R^{12} and R^{17}, R^{17} and R^{19}, R^{17} and R^{21}, R^{19} and R^{21}, R^{19} and R^{13}, or R^{19} and the substituent attached to Z^9 may also be connected to form a ring, and n equals 0 or 1.

7. Chelating compound according to claim 6, wherein -CR^9R^{10}-Z^8-$CR^{17}R^{18}$- represents a heterocyclic group, in particular 2,6-pyridinediyl.

8. Chelating compound according to any one of the preceding claims, wherein Z^5, Z^6 and Z^7 each represent carboxyl and R^1, R^2, R^3, R^4, R^5, R^6, R^7 and R^8 are hydrogen.

9. Chelating compound according to one of the preceding claims, wherein Z^8 represents O or NR^{12}, and Z^9 represents NR^{13}, wherein R^{13} preferably comprises the group A^6-X.

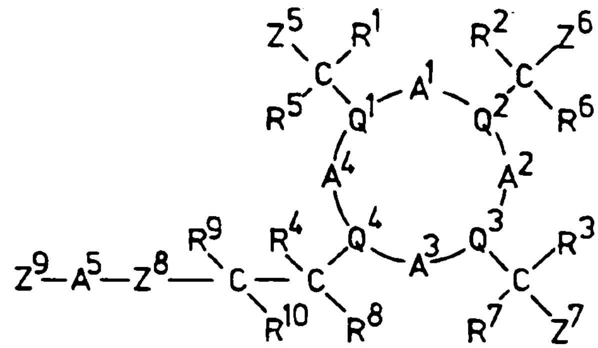
10. Chelating compound according to one of the preceding claims, containing the group A^6-X, wherein X represents isocyanato, isothiocyanato, mercapto, amino, haloacetamido, diazo, succinimido, maleimido, a reactive ester group or an anhydride.

11. Chelate of a chelating compound according to one of the preceding claims and a trivalent metal ion, in particular a radioactive metal.

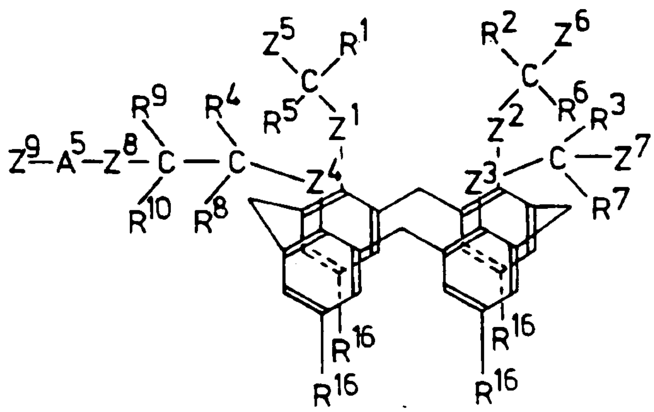
12. Target-specific conjugate comprising a chelating compound according to one of claims 1-11 or a chelate according to claim 10, bound through functional group X to a targeting agent, such as a protein, in particular an antibody.

13. Pharmaceutical composition containing a compound, a chelate, a conjugate according to one of the preceding claims together with a pharmacologically acceptable carrier.

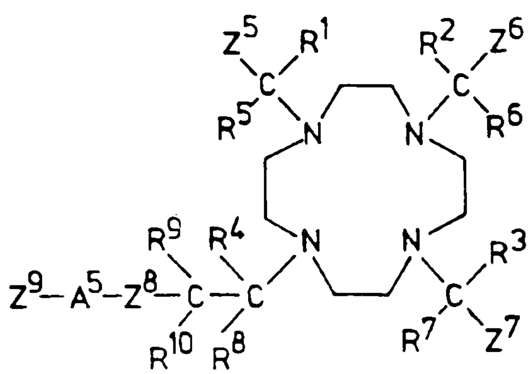
1.


Z5
R1
R2
Z6
C
C
A1
R5
Q1
Q2
R6
A4
A2
R9
R4
Q4
Q3
R3
Z9
A5
Z8
C
C
A3
C
R10
R8
R7
Z7

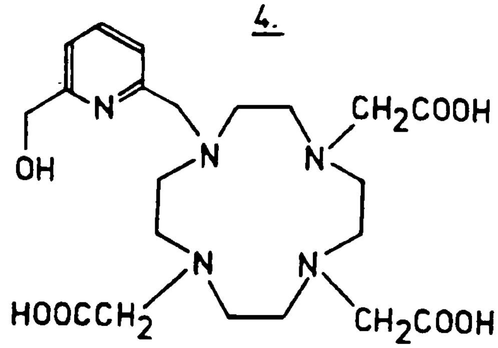
2.


Z5
R1
R2
Z6
C
C
R9
R4
R5
Z1
R6
R3
Z2
Z9
A5
Z8
C
C
Z4
Z3
C
Z7
R10
R8
R7
R16
R16
R16
R16

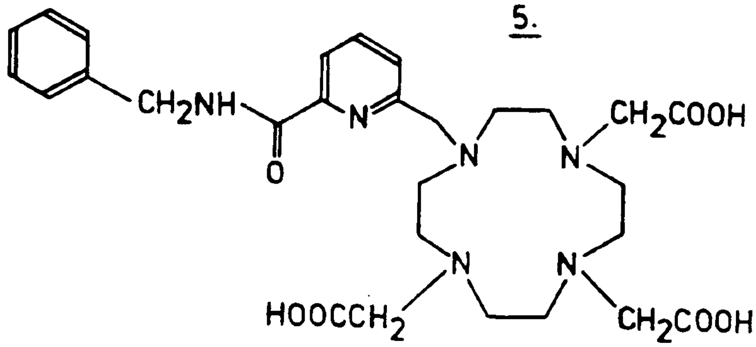
3.


Z5
R1
R2
Z6
C
C
R5
N
N
R6
R9
R4
N
N
R3
Z9
A5
Z8
C
C
C
R10
R8
R7
Z7

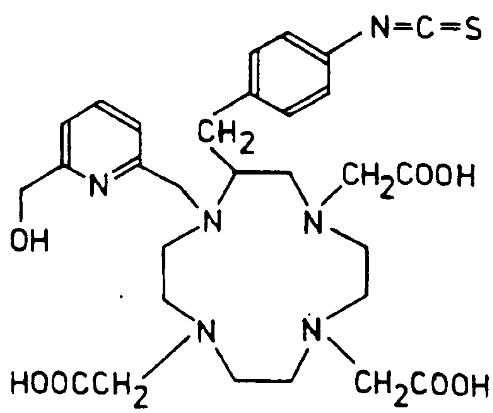
4.



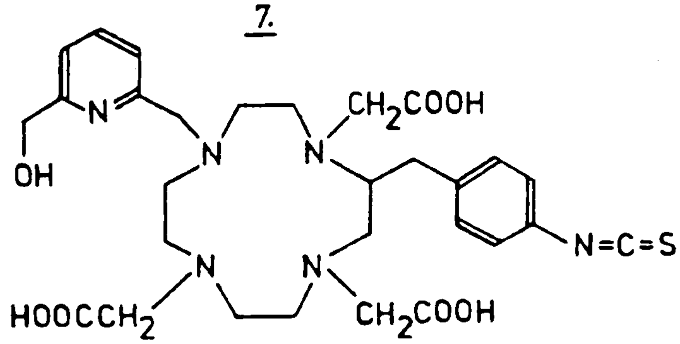
5.



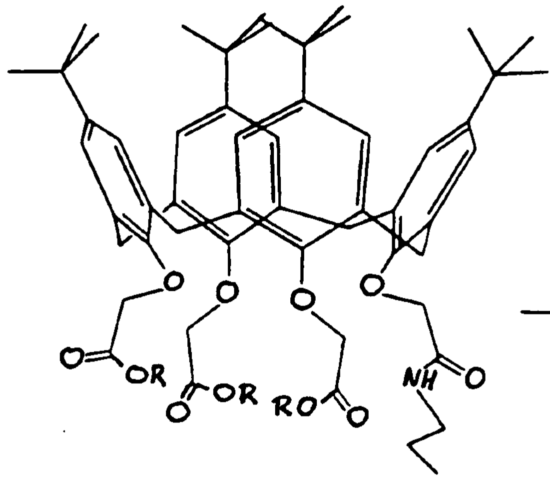
6.



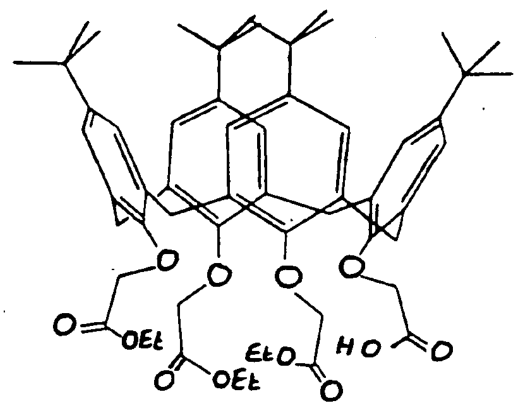
7.


N
OH
CH2COOH
N
N
N=C=S
N
N
HOOCCH2
CH2COOH

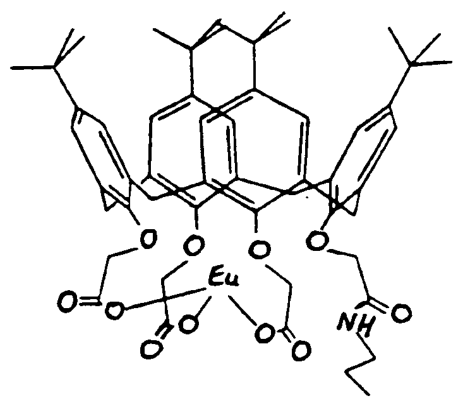
8.


O
O
O
O
OR
OR
RO
NH
O

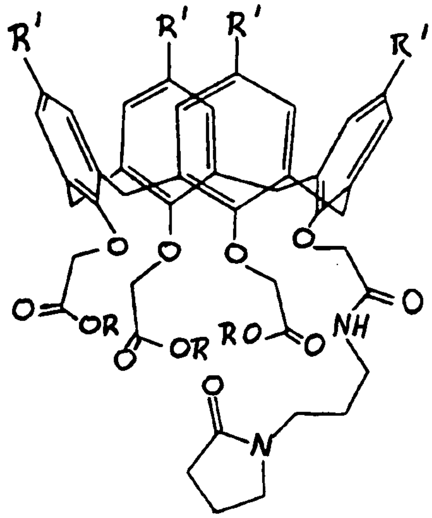
9.


O
O
O
O
OEt
OEt
EtO
HO

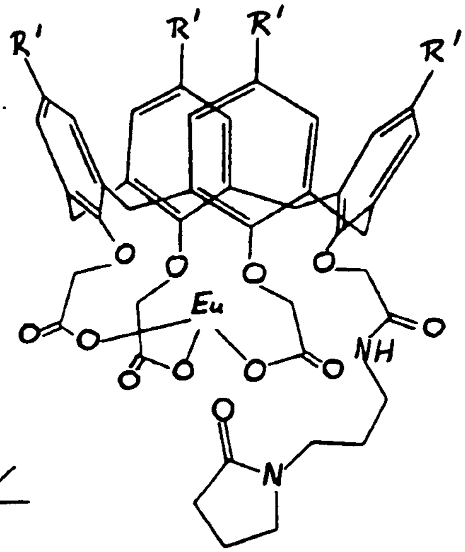
10.


O
O
O
O
Eu
NH

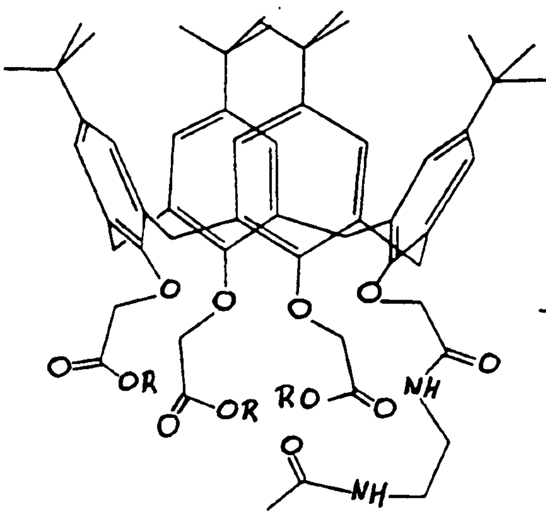
11.



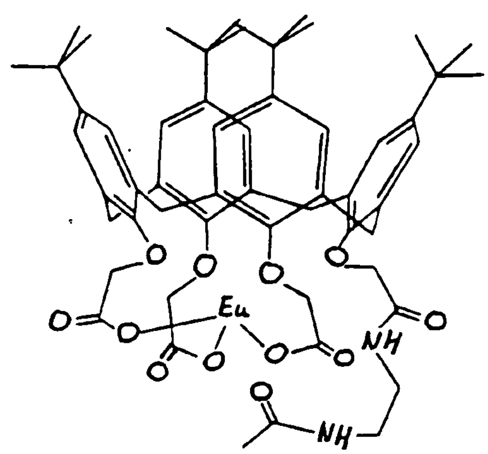
12.



13.



14.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/02126

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D257/02 C07D401/06 C07D207/26 C07C235/06 C07C235/10
A61K51/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07C C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 434 345 (SQUIBB) 26 June 1991 *Document* ---	1-3,6-13
A	EP,A,0 325 762 (BRACCO INDUSTRIA CHIMICA) 2 August 1989 cited in the application *Document* ---	1-3,6-13
A	EP,A,0 434 346 (SQUIBB) 26 June 1991 cited in the application *Document* ---	1-3,6-13
A	WO,A,89 11475 (THE UNITED STATES OF AMERICA) 24 May 1989 cited in the application *Document* --- -/--	1-3,6-13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
10 October 1994	19. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Luyten, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 374 947 (THE DOW CHEMICAL COMPANY) 27 June 1990 cited in the application *Document* ---	1-3,6-11
A	ROYAL SOCIETY OF CHEMISTRY.CHEMICAL COMMUNICATIONS, no.12, 15 June 1990 pages 878 - 879 NANDA SABBATINI ET AL cited in the application *Article* -----	1,4-11

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 94/02126

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0434345	26-06-91	AU-B-	625529	16-07-92
		AU-A-	6704390	27-06-91
		JP-A-	4120065	21-04-92
EP-A-0325762	02-08-89	AU-A-	2904789	19-07-89
		WO-A-	8905802	29-06-89
		EP-A-	0440606	14-08-91
		JP-T-	3501848	25-04-91
		US-A-	5132409	21-07-92
EP-A-0434346	26-06-91	AU-A-	6709390	27-06-91
		JP-A-	4120066	21-04-92
WO-A-8911475	30-11-89	AU-A-	3210893	01-04-93
		AU-B-	630362	29-10-92
		AU-A-	3842889	12-12-89
		EP-A-	0416033	13-03-91
		JP-T-	3503531	08-08-91
EP-A-0374947	27-06-90	US-A-	5006643	09-04-91
		HU-B-	209459	28-06-94
		JP-T-	3502937	04-07-91
		WO-A-	9007342	12-07-90